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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/573, 33/564, 33/574

A1

(11) International Publication Number: WO 95/02824

(43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/EP94/01771

(22) International Filing Date: 31 May 1994 (31.05.94)

(30) Priority Data:
P 43 23 436.4 13 July 1993 (13.07.93) DE
P 43 43 255.7 17 December 1993 (17.12.93) DE

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(81) Designated States: AU, CA, CN, CZ, FI, HU, JP, KR, NO, NZ, PL, SK, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHOD AND KIT FOR THE QUANTITATIVE DETERMINATION OF HUMAN THYROID PEROXIDASE

(57) Abstract

Method for the quantitative determination of human thyroid peroxidase (hTPO) in biological fluids, culture media, tissue extracts and fluids containing highly purified natural hTPO or recombinant hTPO, in which the determination is carried out as a sandwich assay known per se, using a first monoclonal antibody against hTPO and at least one further monoclonal antibody against hTPO (MAB_1, MAB_2), of which the former (MAB_1) recognises hTPO in a region which is sensitive to denaturing and is involved in the binding of the autoantibodies against hTPO and in enzyme inhibition, while the one or more further antibodies (MAB_2) recognises hTPO in a region whose binding properties are not essentially impaired by denaturing of hTPO.

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Method and kit for the quantitative determination of human thyroid peroxidase

The invention relates to a method for the quantitative determination of human thyroid peroxidase (hTPO) in biological fluids, in culture media, in tissue extracts and in fluids containing highly purified natural hTPO or recombinant hTPO, and a kit for carrying out such a method.

Human thyroid peroxidase (designated below as a rule only by the abbreviation hTPO) is a glycosylated haemoprotein which is bound to the thyroid membranes and performs important functions in the biosynthesis of the thyroid hormones. The primary structure of the hTPO was determined after various groups had succeeded in cloning the hTPO gene.

The publication by Jean RUF, Marie-Elisabeth TOUBERT, Barbara CZARNOCKA, Josée-Martine DURAND-GORDE, Mireille FERRAND and Pierre CARAYON, "Relationship between Immunological Structure and Biochemical Properties of Human Thyroid Peroxidase", in Endocrinology, Volume 125, No. 3, pages 1211 to 1218, describes the results

of an investigation to determine the three-dimensional structure of hTPO by mapping its antigenic surface using various monoclonal antibodies (MAB). The stated publication will be referred to several times below, this publication always being referred to briefly as "J. Ruf et al. in Endocrinology 125". The stated publication also contains a review of the literature on the function and determination of the structure of hTPO, reference additionally being made to all publications cited.

It has been found that hTPO is important not only because of its actual function in the biosynthesis of the thyroid hormones but also because it has emerged that hTPO is identical to the so-called microsomal antigen which is recognised as the autoantigen of circulating autoantibodies which can be detected in most patients with autoimmune diseases of the thyroid.

Owing to their great diagnostic importance, a large number of detection methods for autoantibodies against hTPO or the microsomal antigen have already been developed and have become very important in practice. A recent development by the Applicant of the present Application is described in German Patent DE 41 20 412 C1, in which the presence of autoantibodies against hTPO is detected by virtue of the fact that, in the presence of the required antibodies, the formation of a sandwich of a) a first antibody, in particular a monoclonal antibody, b) the antigen hTPO preferably added in crude, natural form and c) a further labelled, preferably likewise monoclonal antibody is disturbed. Regarding further tests for the determination of autoantibodies against hTPO, reference may be made to the introduction of

German Patent DE 41 20 412 C1.

Since the process just described briefly concerns the detection of autoantibodies which, as a rule, occur in high concentrations in the serum of patients suffering from autoimmune diseases of the thyroid, for the functioning of the stated tests it was essential that a sandwich whose formation is disturbed by the presence of autoantibodies against hTPO is formed in a sufficiently reproducible way from the two antibodies and the crude, natural antigen added in relatively high concentrations. For the test described, it was not necessary for the formation of the sandwich disturbed by the presence of the required antibodies to take place with high sensitivity.

The present invention is based on the surprising finding that it is possible, using two monoclonal antibodies such as those which can be used in the method for the determination of autoantibodies against hTPO according to DE 41 20 412 C1, to carry out a highly sensitive measurement of the antigen hTPO in various biological fluids and in particular in tissue extracts, in culture media and similar fluids in laboratory practice.

According to Claim 1, the present invention therefore relates to a method for the quantitative determination of human thyroid peroxidase (hTPO) in biological fluids, culture media, tissue extracts and fluids containing highly purified natural hTPO or recombinant hTPO, characterised in that the determination is carried out as a sandwich assay known per se, using a first monoclonal antibody against hTPO and at least one further monoclonal antibody against hTPO (MAB_1, MAB_2),

of which the former (MAB_1) recognises hTPO in a region which is sensitive to denaturing and is involved in the binding of the autoantibodies against hTPO and in enzyme inhibition, while the one or more further antibodies (MAB_2) recognises hTPO in a region whose binding properties are not impaired by denaturing of hTPO.

Preferred embodiments of the stated method and the basic composition of preferred kits for carrying out such a method are described in Claims 2 to 11.

The present invention, including its background and its particular embodiments and advantages, is described in detail below.

While the detection of autoantibodies against hTPO has been the subject of extensive research and development work, there is so far no reliable and commercially available method for the detection of hTPO in biological fluids, culture media and other biological materials in the widest sense of this term.

The only description of a direct measurement of hTPO found in a literature search appears in T.J. Wilkin and J.L. Diaz "Approaches to the measurement of TPO in serum", in: Thyroperoxidase and Thyroid Autoimmunity, Ed. P. Carayon, J. Ruf. Colloque INSERM/John Libbey Eurotext Ltd., 1990, Vol. 207, pages 169-172. In the stated work, an attempt is made to determine the content of circulating hTPO. It is reported that attempts to develop an enzymatic test based on a guajacol assay were unsuccessful. The authors therefore carried out their measurements using a radioimmunoassay in which purified hTPO labelled with

^{125}I was reacted with a polyclonal rabbit anti-hTPO serum to give immune complexes which were precipitated by the double antibody method by adding a donkey anti-rabbit globulin.

To determine the hTPO content, the degree of displacement of the labelled hTPO from the precipitated immune complexes was determined. The method described has a low sensitivity of about 2 ng/ml, and the hTPO serum concentrations determined by the stated method were so high that there is good reason for doubting the correctness of the values obtainable by the stated method. The reason for measuring hTPO concentrations in the serum was the desire to test the correctness of various theories on the formation of autoantibodies against hTPO in autoimmune diseases of the thyroid. One of these theories assumes that the formation of autoantibodies is due to the fact that endogenous substances normally fixed in the cell or cell membrane, for example the enzyme hTPO, is able to enter the blood circulation as a result of damage to the cell or cell membrane and to cause in said circulation a "normal" immune reaction of the body to antigens not normally present in the circulation.

In the work by U. Feldt-Rasmussen, M. Hoier-Madsen, J. Date and M. Blichert-Toft in the same publication: Thyroperoxidase and Thyroid Autoimmunity, page 173, an attempt is also made to draw conclusions about the concentration of hTPO in the serum after an operation on the thyroid gland. In the absence of a suitable method for determining hTPO, the measurable concentration of anti-hPTO autoantibodies was determined and the decrease in the measurable concentration was correlated with an equivalent release

of hTPO which reduces its measurable concentration via binding of autoantibodies. The indirect determination of circulating hTPO for the reduction of the autoantibody concentration was carried out because tests for determining such antibodies were available but there was no reliable test for the direct determination of hTPO concentrations. However, every indirect determination has the disadvantage that it is based on a very large number of assumptions which cannot be checked and greatly limit the value of the results obtained.

The two last-mentioned papers which describe the attempt to determine hTPO in biological fluids give various reasons as to why it may be of scientific and clinical interest to be able to measure not only autoantibodies against hTPO but also hTPO itself by a direct method. Further areas in which a reliable method for the determination of the key enzyme hTPO would be helpful are in vitro investigations using biological samples, including cell cultures (primary cultures, constant cell cultures), since in this area it may be important to determine whether the cell cultures contain a peroxidase, such as hTPO. Thus, it is also known, for example, that peroxidases play an important role in the metabolism of chemicals and may be substantially involved in the formation of certain metabolites which can be important, for example, for the toxicity of certain substances. Furthermore, a reliable method for the direct determination of hTPO can also be used for concentration determination or calibration and standardisation of hTPO contents in commercial products or in products and intermediates obtained by genetic engineering.

The method according to the invention provides for the first time a method which permits a highly sensitive measurement of the antigen hTPO by the sandwich assay known per se. In the method, a monoclonal anti-hTPO antibody is fixed, in agreement with the principle known per se, on a solid phase, preferably on the wall of coated polystyrene tubes, and a highly sensitive hTPO assay (lower limit of detection about 26 pg TPO/ml) with highly dynamic characteristics (measuring range more than three orders of magnitude) and high reproducibility (mean interassay coefficient of variance 3.6%) is provided with the use of a further labelled anti-hTPO antibody which is preferably labelled with an acridiniumester and has chemiluminescent properties.

In the method according to the invention, two antibodies which can also be used in the process according to DE 41 20 412 C1 and which correspond to the antibodies described in J. Ruf et al., Endocrinology 125, and from the clones with the numbers 15 and 53 (referred to below as MAB 15 and MAB 53 for short) are preferably used. For completion of the disclosure of the present application, the two preferably used antibodies were deposited as a precaution, in the form of the hybridoma cells producing these antibodies, in "DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Maschenroder Weg 1b, Braunschweig, Germany, in accordance with the Budapest Agreement. The deposition numbers are DSM ACC2154 (acceptance date 14.09.1993) for the hybridoma with the designation "TPO # 15-4-G2" and DSM ACC2137 (acceptance date 07.07.1993) for the hybridoma "TPO # 53".

As explained in the publication by J. Ruf et al., Endocrinology 125, MAB 15 is one of the antibodies which binds hTPO in a region which is also involved in the binding of autoantibodies against hTPO and furthermore in enzyme inhibition. Since the binding of the MAB 15 to hTPO is greatly reduced under conditions which make it likely that denaturing of hTPO will occur, it may be concluded that the stated monoclonal antibody recognises a certain conformation of hTPO (i.e. is a so-called conformation antibody). On the other hand, the MAB 53 (J. Ruf et al., Endocrinology 125) preferably used as a further labelled monoclonal antibody is one of the antibodies whose binding is impaired to a very much lesser extent by denaturing of the hTPO, so that it is assumed that this monoclonal antibody tends to recognise a region of the primary amino acid sequence and is therefore a so-called sequential antibody.

Although the use of the two stated antibodies leads to an outstanding method for the determination of hTPO, it is however within the scope of the present invention to use one or more labelled monoclonal or polyclonal antibodies with comparable properties instead of the labelled MAB 53 or in addition thereto, i.e. sequential antibodies in the above sense which do not interfere with the binding of hTPO to an MAB 15. In particular, the MAB 53 can also be used together with one of the monoclonal antibodies 30 to 47 (J. Ruf et al., Endocrinology 125) or can be replaced by a mixture of the two stated monoclonal antibodies. The use of a mixture of two of the stated monoclonal antibodies is of particular interest in connection with the paper by C. de Micco, J. Ruf, M.A. Chrestian, N. Gros, J.F. Henry and P. Carayon, likewise in the above-mentioned

publication Thyroperoxidase and Thyroid Autoimmunity, Vol. 207, pages 133-136. In the stated publication, the authors show that the monoclonal antibodies 30 and 47 bind to the hTPO of tissue samples in different ways, depending on whether the tissue samples originate from healthy tissue or a benign tumour or whether they originate from a malignant tumour. By using two monoclonal antibodies which have different types of labels or permit a distinction between the individual labels as a mixture in the method according to the invention for the quantitative determination of hTPO, it is possible, for example, to draw conclusions about the state of health of the tissue donor from the uniform or different binding of the two antibodies and hence also of the two different labels.

Although this stated procedure is clearly intended also to be covered by the present invention, it will not be discussed further below. The preferred method for the determination of hTPO using monoclonal antibodies 15 and 53 according to the stated publication by J. Ruf et al., Endocrinology 125 (deposition designations TPO # 15-4-G2 and TPO # 53, respectively, at DSM) will be described in detail below.

In the Figures,

Fig. 1 shows the results of the hTPO measurement by the method according to the invention in different measuring matrices,

Fig. 2 shows the specific hTPO values measured by the method according to the invention in the case of different tissue extracts and

Fig. 3 shows the effect of the presence of leupeptin on the reproducibility of the hTPO measured in tissue extracts with increasing age of the samples.

Example

1. Immobilisation of a monoclonal anti-hTPO antibody on a solid phase:

The antibody deposited at DSM under the deposition designation TPO # 15-4-G2, corresponding to the antibody MAB 15 (J. Ruf et al., Endocrinology 125), was chosen as the antibody which is coupled to the solid phase. The coupling of the stated monoclonal antibody to a solid phase was carried out by coating a polystyrene tube by a known method, as follows:

Polystyrene test tubes having dimensions of 12 x 75 mm (obtained from the company Greiner) were each filled with 1 µg of the anti-hTPO antibody 15 in 300 µl of an aqueous buffer solution (10 mM tris HCl; 10 mM sodium chloride; pH 7.8). After incubation for 20 hours at room temperature, the tubes were washed twice (4.5 ml of H_2O each time). The tubes were then saturated with a solution of 0.5% BSA (bovine serum albumin) by filling them with the saturation solution, incubating them for two hours at room temperature and then emptying them by decanting the content. The tubes were then freeze-dried with the applied coating. In this form, the ready-to-use tubes were included in the test kit for the method.

2. Preparation of an anti-hTPO antibody labelled with a chemiluminescence label:

150 µg of the antibody which was purified by affinity chromatography, had been deposited at DSM under the deposition designation TPO # 53 and corresponds to the antibody MAB 53 (J. Ruf et al., Endocrinology 125), protein concentration 1 mg/ml in 20 mM sodium phosphate, pH 7.4, were reacted with 25 nmol of acridinium ester (active ester). After an incubation time of 30 min, unbound free label was separated from the labelled antibody by HPLC over a WATERS-Shodex WS 803 column (flow rate 1 ml/min, mobile phase 150 mM sodium phosphate, pH 7.2).

The purified tracer obtained in this manner is diluted to a total activity of 1 x 10^8 RLU (relative light units) per ml in 50 mM Hepes buffer, 1% BSA (from MILES) and 1 mg/ml mouse IgG (from SCANTIBODIES), pH 6.5, and is filled in portions of 0.5 ml into 10 ml amber glass bottles and then freeze-dried.

The freeze-dried tubes were included in the kit. Before an anti-hTPO test is carried out, the tracer is reconstituted in each case with 5 ml of a buffer having the following composition: 50 mM Hepes, 100 mM sodium chloride, 0.5% Triton X100 (from PIERCE), pH 6.5, which is also part of the test kit.

3. Preparation of the standard or calibrators:

Various calibrators can be used:
a) Very pure thyroid peroxidase, isolated from human thyroid membranes and purified by affinity chromatography (cf. J. Ruf et al., Endocrinology).

b) Recombinant human thyroid peroxidase (purified by affinity chromatography), which is available from the company WBAG Resources/Zürich, with an hTPO concentration of 36.96 µg/ml in a buffer solution of 25 mM tris HCl (pH 7.4) containing 0.1 M KI.

c) Crude thyroid peroxidase which is isolated from human thyroid and prepared according to P 41 20 412 C1 and the content of which is calibrated using a) or b).

For the preparation of the calibrators, hTPO is diluted to the appropriate concentrations as a five-fold concentrate in PBS (phosphate-buffered saline solution) + 1% BSA and 0.6% Triton X100 (from PIERCE) and filled in portions of 0.2 ml each into 2 ml glass bottles and then freeze-dried. The calibrators are used by reconstituting them to a volume of 1.0 in the medium in which the desired measurement is to be carried out (e.g. serum or buffer).

The measurement is carried out by a procedure in which 200 µl of the standard or of the sample are pipetted into the tubes coated with the immobilised monoclonal antibody and then 100 µl of tracer are pipetted in each case. After an incubation time of 16 to 20 hours at room temperature under the strict exclusion of light owing to the light sensitivity of the luminescence label, 1 ml of wash solution is added to each tube, after which the tube content is decanted. Washing is then carried out three times with 1 ml of wash solution each time, followed each time by decantation. The tubes are then placed for 5 to 10 minutes with the open side facing downwards on blotting paper in order to suck up the residual fluid. All coated tubes are then placed in a luminometer for measurement of the

chemiluminescence signal, the required reagents being added in a known manner, preferably automatically, and the luminous efficiency being measured in a period of 1 s.

Results:

Using the reagent set described and the stated measurement protocol, amounts of tracer which increased with increasing amounts of hTPO were bound to the tube surface. It was found that the resulting test has a high dynamic range which extends over three orders of magnitude (linear measured signal of 0.02 to over 50 ng TPO/ml).

As is to be expected, the shape of the standard curve is influenced by the measurement media used, Fig. 1 showing the standard curves obtained for the measurement media buffer, medium + 10% FCS, serum, saliva, plasma and urine.

An important advantage of the design of the present method for the user is that the calibrators can be reconstituted in any desired measurement medium, so that there is complete freedom with regard to the choice of the so-called measurement matrix. In the method according to the invention, the natural hTPO purified by affinity chromatography and recombinant hTPO purified by affinity chromatography give identical results in the measurements.

The sensitivity (lower limit of detection of the test) was determined as 26 pg hTPO/ml (mean value of the RLU values of the reference standard plus 3 standard deviations in buffer matrix), a surprisingly good

value.

Thus, using the test described for carrying out the method according to the invention, it is possible to determine hTPO with high sensitivity, dynamic range and selectivity in virtually any medium.

However, it should be pointed out that, in a determination in biological fluids, e.g. serum, in which the presence of auto-anti-hTPO antibodies must also be expected, a so-called recovery test for the presence of autoantibodies must be carried out. If necessary, antibodies can also be removed from such a sample in a step preceding the actual measurement method. Furthermore, it is within the scope of the present invention to modify the test kit described so that the antibodies used are immobilised or labelled in a reverse order and the method is carried out with an intermediate wash step since the monoclonal antibody 53 binds hTPO but does not react with corresponding autoantibodies.

In a measurement of hTPO in thyroid tissue extracts, it is also advisable, in order to obtain reproducible results, to carry out the hTPO determination in the presence of an endoprotease inhibitor, in particular of a leupeptin (cf. following Example of Use).

Example of Use

Determination of the amount of hTPO in various human thyroid tissue extracts

Procedure

Immediately after the surgical removal of the tissue,

the samples are frozen on solid carbon dioxide and stored at minus 20°C until required for further use. After thawing of the tissue, the material is weighed and is homogenised with 10 times the weight of PBS buffer, which additionally contains 0.5% Triton X100 and 500 µm leupeptin (Ultra-Turrax homogeniser, IKA-Werke/Staufen; 5 times at a maximum speed of 10 s in each case). After incubation for 30 minutes, the samples are centrifuged for one hour at 100,000 g, and the resulting supernatant solution is measured to determine its protein content and hPTO content. The resulting quotient of the amount of hTPO and the amount of the protein is shown in Fig. 2.

As shown in Fig. 2, the specific hTPO content of thyroid tissue or operation materials is subjected to drastic changes. Even with the small number of samples shown, differences in the specific content of up to a factor of 8 are found.

It should also be pointed out that it had been found, surprisingly, that, when measured by the hTPO assay according to the invention, the hTPO content in thyroid extracts increases drastically with time during storage at 4°C. A leupeptin-sensitive protease appears to be responsible for this effect, since leupeptin practically completely suppresses this effect (cf. Fig. 3), and, in order to obtain reproducible results, the determination of the hTPO in tissue extracts must therefore be carried out in the presence of leupeptin. Interestingly, it has also been found that the rate of hTPO increase is highly dependent on the individual tissue (data not shown).

Figure 3 shows the results of experiments in which the TPO extract was stored at room temperature with and without the addition of 500 µM of leupeptin. The extracts were measured at different time intervals using TPO standard series. For this purpose, they were diluted in the standard buffer.

Claims

1. Method for the quantitative determination of human thyroid peroxidase (hTPO) in biological fluids, culture media, tissue extracts and fluids containing highly purified natural hTPO or recombinant hTPO, characterised in that the determination is carried out as a sandwich assay known per se, using a first monoclonal antibody against hTPO and at least one further monoclonal antibody against hTPO (MAB_1, MAB_2), of which the former (MAB_1) recognises hTPO in a region which is sensitive to denaturing and is involved in the binding of autoantibodies against hTPO and in enzyme inhibition, while the one or more further antibodies (MAB_2) recognises hTPO in a region whose binding properties are not essentially impaired by denaturing of hTPO.

2. Method according to Claim 1, characterised in that the first monoclonal antibody (MAB_1) is bound in immobilised form to a solid phase and is used in excess relative to the hTPO concentration expected in the sample to be investigated, and that the one or more monoclonal antibodies (MAB_2) are labelled.

3. Method according to Claim 2, characterised in that the one or more further monoclonal antibodies (MAB_2) are labelled with a chemiluminescence label, a radio isotope, an enzyme label or a fluorescence label.

4. Method according to Claim 3, characterised in that the chemiluminescence label is an acridinium ester.

5. Method according to Claim 3 or 4, characterised

in that, in addition to the one or more labelled monoclonal antibodies, an additional labelled monoclonal or polyclonal antibody against hTPO is present and can be distinguished from the one or more labelled antibodies by means of its different label and furthermore differs from one or more said labelled antibodies by virtue of the fact that it binds hTPO in a different region and/or with a different affinity.

6. Method according to any of Claims 1 to 5, characterised in that the first monoclonal antibody (MAB_1) is a monoclonal antibody which is produced by the hybridoma deposited under the designation TPO # 15-4-G2 (DSM ACC2154), and that the one further monoclonal antibody or one of the further monoclonal antibodies is a monoclonal antibody (MAB_2) which is produced by the hybridoma deposited under the designation TPO # 53 (DSM ACC2137).

7. Method according to either of Claims 5 or 6, characterised in that it is determined whether the further, differently labelled antibodies are bound in a similar way or in greatly differing ways, and that conclusions about the presence or absence of a malignant disease are drawn therefrom.

8. Method according to any of Claims 1 to 7, characterised in that a protease inhibitor for endoproteases is added to human tissue extracts to be assayed for hTPO after said extracts have been obtained.

9. Method according to Claim 8, characterised in that leupeptin is used as the protease inhibitor.

10. Kit for carrying out a method according to any of the preceding Claims, characterised in that it contains a solid phase having a first monoclonal antibody against hPTO (MAB_1) in freeze-dried form, at least one further labelled antibody (MAB_2), preferably likewise in freeze-dried form, and a calibrator in freeze-dried form and containing defined amounts of hTPO, and customary buffers, solvents and, if required, reagents for the detection of the label.

11. Kit according to Claim 10, characterised in that it contains, as the calibrator, i) highly purified hTPO from human thyroid membranes, ii) recombinant hTPO or iii) crude hTPO isolated from human thyroids and calibrated against i) or ii).

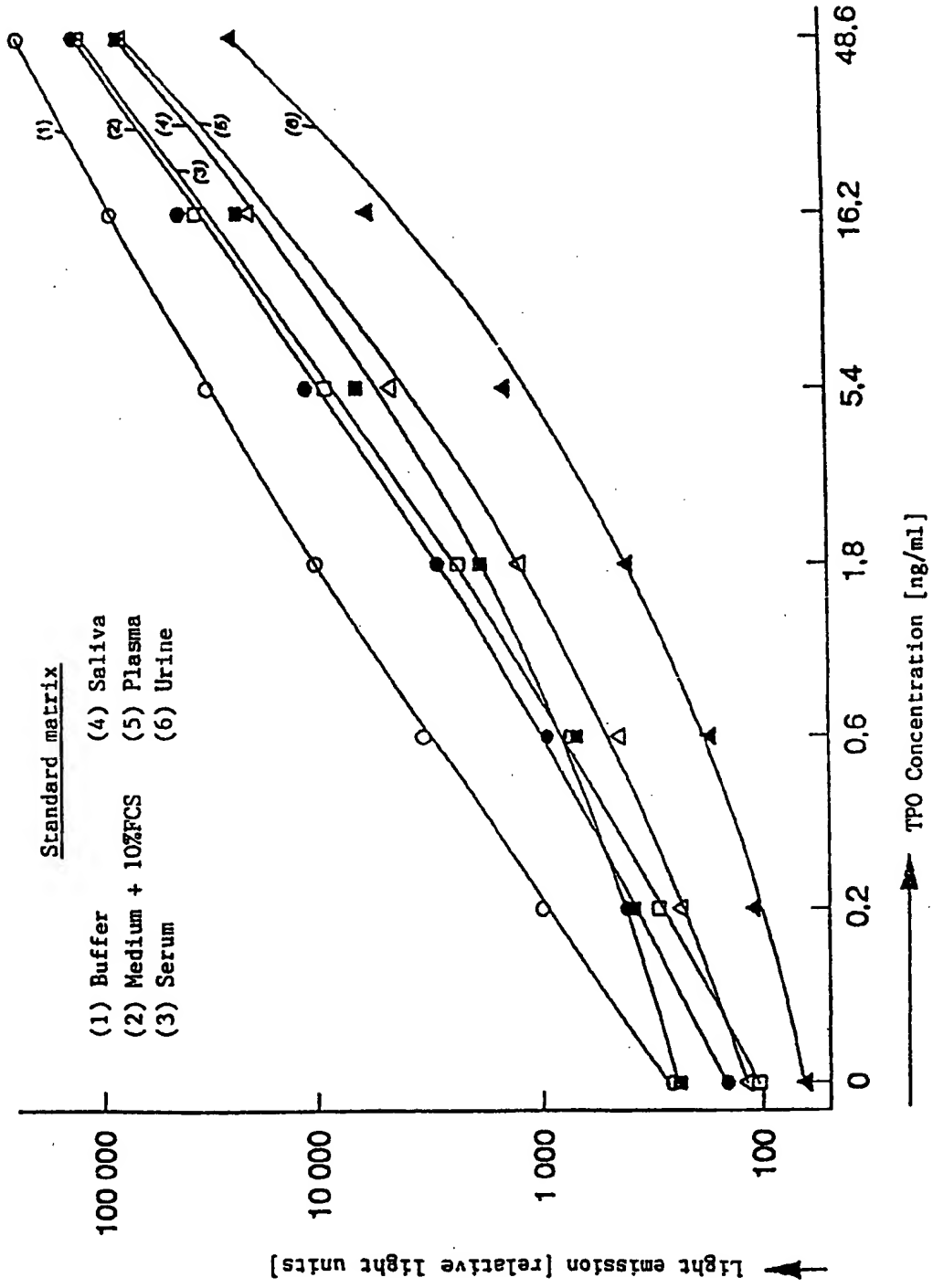
Light emission [relative light units]
100 000
10 000
1 000
100
TPO Concentration [ng/ml]
0
0,2
0,6
1,8
5,4
16,2
48,6
Standard matrix
(1) Buffer
(2) Medium + 10%FCS
(3) Serum
(4) Saliva
(5) Plasma
(6) Urine
(1)
(2)
(3)
(4)
(5)
(6)

FIG. 1

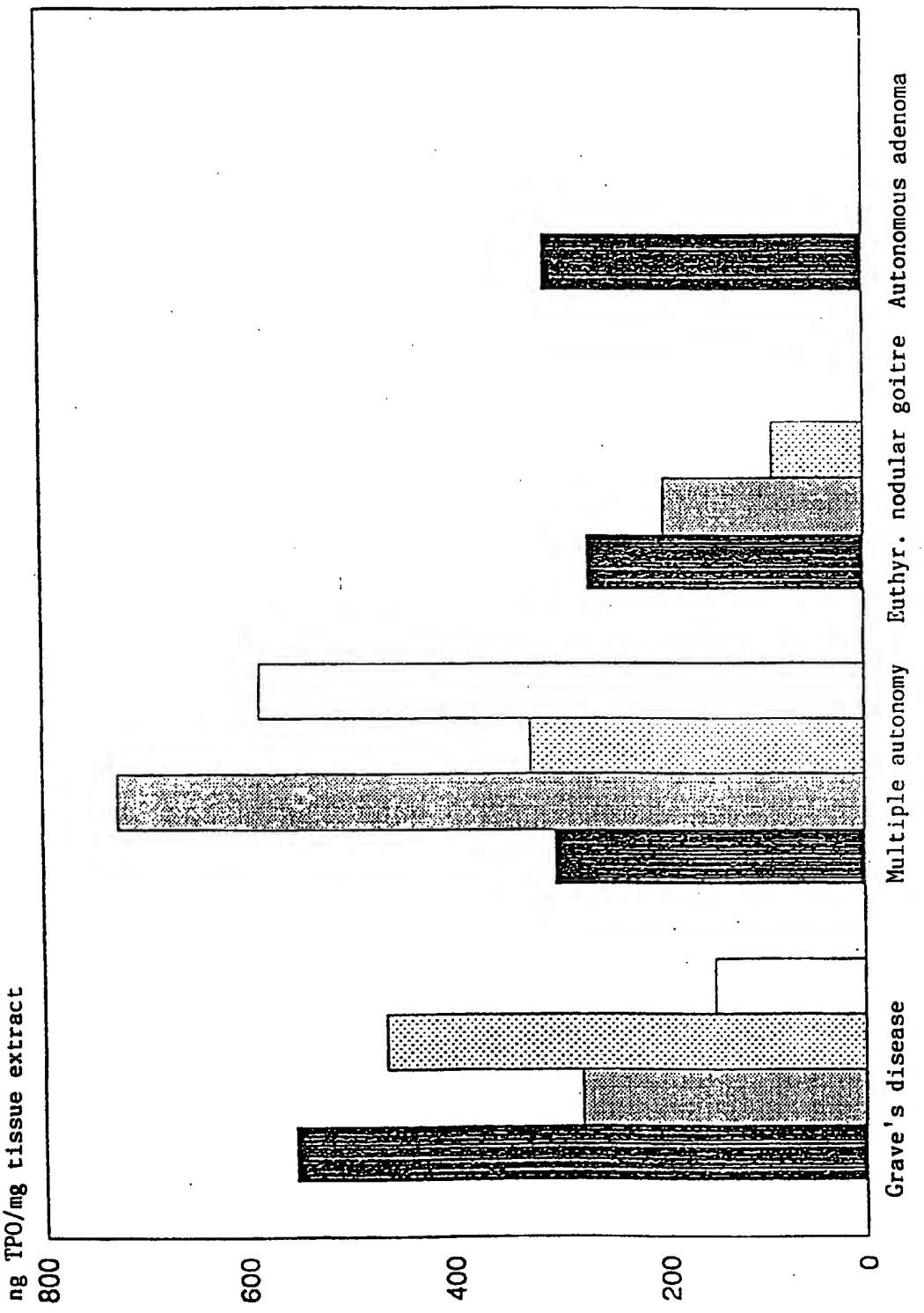
ng TPO/mg tissue extract
800
600
400
200
0
Grave's disease
Multiple autonomy
Euthyr. nodular goitre
Autonomous adenoma

FIG. 2

Stability of the TPO in the thyroid extract

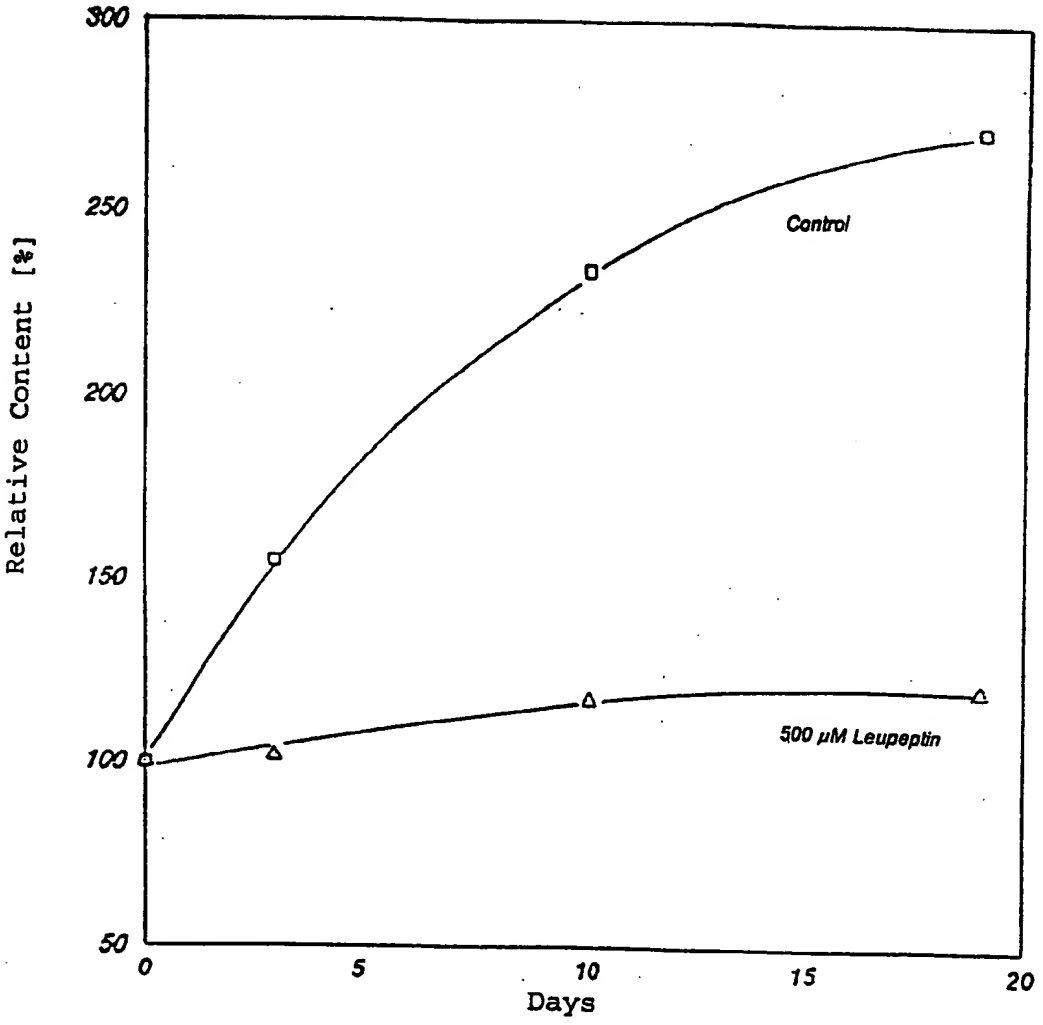

FIG. 3

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/EP 94/01771

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/573 G01N33/564 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE,A,41 20 412 (HENNING BERLIN GMBH CHEMIE UND PHARMAWERK) 7 January 1993 cited in the application See the abstract; Column 9, line 60- column 10, line 6; claims. ---	1-4,10
A	ENDOCRINOLOGY, vol.125, no.3, 1989 pages 1211 - 1218 J.RUF ET AL. 'Relationship between Immunological Structure and Biochemical Properties of Human Thyroid Peroxidase' cited in the application see the whole document --- -/--	1,5,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 September 1994	26.09.94

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hitchen, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THYROPEROXIDASE AND THYROID AUTOIMMUNITY, vol.207, 1990 pages 169 - 172 T.J.WILKIN ET AL. 'Approaches to the measurement of TPO in serum' cited in the application see the whole document ---	1,5,7
A	WO,A,91 02061 (B.RAPAPORT) 21 February 1991 see page 26 - page 32; claims -----	1-3,10

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/EP 94/01771

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		EP-A-	0544869	09-06-93
		JP-T-	6501103	27-01-94
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